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Final Screening Assessment

Acetamide, N-(4-ethoxyphenyl)- (Phenacetin)

Chemical Abstracts Service Registry Numbers 62-44-2

Environment and Climate Change Canada
Health Canada

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Synopsis

Pursuant to section 74 of the Canadian Environmental Protection Act, 1999 (CEPA), the Minister of the Environment and the Minister of Health have conducted a screening assessment of acetamide, N-(4-ethoxyphenyl)-, hereinafter referred to as phenacetin. The Chemical Abstracts Service Registry Number (CAS RN[1]) for phenacetin is 62-44-2. This substance is among those substances identified as priorities for assessment as it met categorization criteria under subsection 73(1) of CEPA.

In 2008, there were no reports of manufacture or import above the reporting threshold of 100 kg in Canada, although it was reported as being imported into Canada in quantities below or equal to the reporting threshold. Phenacetin was formerly used as an analgesic and antipyretic but has not been used in Canada as a therapeutic agent since 1973. It is used primarily as a laboratory reagent, and in a small number of oxidative hair dye preparations, where it functions as a stabilizer for hydrogen peroxide.

The ecological risk of phenacetin was characterized using the Ecological Risk Classification of organic substances (ERC). The ERC is a risk-based approach that employs multiple metrics for both hazard and exposure based on weighted consideration of multiple lines of evidence for determining risk classification. Hazard profiles are established based principally on metrics regarding mode of toxic action, chemical reactivity, food web-derived internal toxicity thresholds, bioavailability, and chemical and biological activity. Metrics considered in the exposure profiles include potential emission rate, overall persistence, and long-range transport potential. A risk matrix is used to assign a low, moderate or high level of potential concern for substances based on their hazard and exposure profiles. The ERC identified phenacetin as having low potential to cause ecological harm.

Considering all available lines of evidence presented in this screening assessment, there is low risk of harm to organisms and the broader integrity of the environment from phenacetin. It is concluded that phenacetin does not meet the criteria under paragraphs 64(a) or (b) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

For the general population of Canada, potential exposures to phenacetin were estimated from dermal contact with the scalp during the use of hair dyes.

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The critical effect for risk characterization was determined to be carcinogenicity, based principally on the International Agency for Research on Cancer (IARC) conclusion that there is sufficient evidence that phenacetin is carcinogenic to humans and experimental animals. Non-cancer effects, including nephropathy and haematotoxicity, have also been observed in humans and laboratory studies. Margins between estimates of exposure and critical effect levels observed in animal studies are considered adequate to address uncertainties in the health effects and exposure databases for both cancer and non-cancer endpoints.

Based on the information presented in this screening assessment, it is concluded that phenacetin does not meet the criteria under paragraph 64(c) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

Therefore, it is concluded that phenacetin does not meet any of the criteria under section 64 of CEPA.

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1. Introduction

Pursuant to section 74 of the Canadian Environmental Protection Act, 1999 (CEPA) (Canada 1999), the Minister of Environment and the Minister of Health have conducted a screening assessment of acetamide, N-(4-ethoxyphenyl)-(herein referred to as phenacetin). This substance was identified as a priority for assessment under Canada's Chemicals Management Plan (CMP), because it met categorization criteria under subsection 73(1) of CEPA (ECCC, HC [modified 2007]).

The ecological risk of phenacetin was characterized using the Ecological Risk Classification of organic substances (ERC) (ECCC 2016a). The ERC describes the hazard of a substance using key metrics including mode of action, chemical reactivity, food-web derived internal toxicity, bioavailability, and chemical and biological activity and considers the possible exposure of organisms in the aquatic and terrestrial environments based on factors including potential emission rates, overall persistence and long-range transport potential in air. The various lines of evidence are combined to identify substances as warranting further evaluation of their potential to cause harm to the environment or as having a low likelihood of causing harm to the environment.

The substance currently being evaluated was previously reviewed internationally through the International Agency for Research on Cancer (IARC) Monographs Programme and there is a recent (2012) IARC Monograph available. These assessments undergo rigorous review and endorsement by international government authorities. Health Canada considers these assessments as reliable. IARC monograph 100A 'Phenacetin' was used to inform this assessment. The U.S. EPA (2002) has also assessed phenacetin and concluded it is a "probable human carcinogen". Likewise, the U.S. National Toxicology Program has concluded that phenacetin is "reasonably anticipated to be a human carcinogen" (U.S. NTP, 2014).

This screening assessment includes consideration of information on chemical properties, environmental fate, hazards, uses and exposure, including additional information submitted by stakeholders. Relevant data were identified and targeted literature searches were conducted up to March 2016; additional information was submitted by Health Canada programs up to October 2017. Empirical data from key studies as well as some results from models were used to reach the conclusions. When available and relevant, information presented in assessments from other jurisdictions was considered.

This screening assessment was prepared by staff in the Consumer Product Safety Program at Health Canada and the CEPA Risk Assessment Program at Environment and Climate Change Canada and incorporates input from other programs within these departments. The ecological portion of this assessment is based on the ERC document (published July 30, 2016), which was peer-reviewed and subject to a 60-day public comment period. The human health portion of this assessment has undergone external review and/or consultation. Comments on the technical portions relevant to human health were received from Dr. John Reichard (Department of Environmental Health,

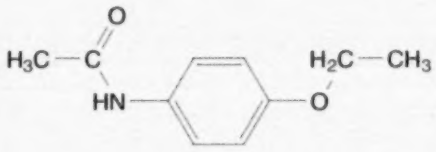
College of Medicine, University of Cincinnati), Dr. Jennifer Sahmel (Cardno Chemrisk), and Dr. Patricia McGinnis (York & Associates). Additionally, the draft of this screening assessment (published April 15, 2017) was also subject to 60-day public comment period. While external comments were taken into consideration, the final content and outcome of the Screening Assessment remain the responsibility of Environment and Climate Change Canada and Health Canada.

This screening assessment focuses on information critical to determining whether a substance meets the criteria as set out in section 64 of CEPA, by examining scientific information and incorporating a weight of evidence approach and precaution[2]. The final screening assessment presents the critical information and considerations upon which the conclusion was made.

2. Identity of substance

The Chemical Abstracts Service Registry Number (CAS RN[3]), Domestic Substances List (DSL) name and common name for this substance are presented in Table 2-1.

Table 2-1. Substance identity

CAS RN	DSL name [common name]	Chemical structure and molecular formula	Molecular weight (g/mol)
62-44-2	Acetamide, N-(4-ethoxyphenyl) [phenacetin]	 $C_{10}H_{13}NO_2$	179.2

Synonyms: Acetamide, N-(4-ethoxyphenyl)-; p-Acetophenetidide; 4'-Ethoxyacetanilide; 4-(Acetylamino)phenetole; 4-Ethoxy-1-acetylaminobenzene; 4-Ethoxyacetanilide; Aceto-4-phenetidine; Acetophenetidin; Acetophenetidine; Acetophenetin;

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[2]A determination of whether one or more of the criteria of section 64 of CEPA are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposures in the general environment. For humans, this includes, but is not limited to, exposures from ambient and indoor air, drinking water, foodstuffs, and products used by consumers. A conclusion under CEPA is not relevant to, nor does it preclude, an assessment against the hazard criteria specified in the Hazardous Products Regulations which are part of the regulatory framework for the Workplace Hazardous Materials Information System for hazardous products intended for workplace use, handling and storage. Similarly, a conclusion based on the criteria contained in section 64 of CEPA does not preclude actions being taken under other sections of CEPA or other Acts.

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Acetparaphenetidine; Acetphenetidin; N-(4-Ethoxyphenyl)acetamide; N-Acetyl-4-ethoxyaniline; N-Acetyl-p-ethoxyaniline; N-Acetyl-p-phenetidine; p-Ethoxyacetanilide; Phenacetine; Phenidin; Phenin (STN, 2016); 1-Acetamido-4-ethoxybenzene; Acet-p-phenalide; Acetanilide, 4'-ethoxy-; Acetic acid amide, N-(4-ethoxyphenyl)-; Aceto-para-phenalide; Aceto-para-phenetidide; Acetylphenetidin; N-Acetyl-para-phenetidine; N-para-Ethoxyphenylacetamide; p-Acetophenetide; p-Acetophenetidine; p-Acetphenetidin; p-Phenetidine, N-acetyl-; para-Acetophenetidide; para-Acetophenetidine; para-Acetphenetidin; para-Ethoxyacetanilide; para-Phenacetin; Paracetophenetidin; Phenacet; Phenacetinum; Phenacitin; Phenazetina (ChemIDplus, 2016).

3. Physical and chemical properties

A summary of physical and chemical properties of phenacetin is presented in Table 3-1. When experimental information was limited or not available for a property, data from (Quantitative) Structure-Activity Relationship ((Q)SAR) models were used to generate predicted values for the substance. Additional physical and chemical properties are presented in ECCC (2016b).

Table 3-1. Experimental or estimated physical and chemical property values (at standard temperature and pressure) for phenacetin

Property	Value	Key reference
Physical state	odorless, white, glistening crystals, usually scales or as fine white, crystalline powder	Osol (1980)
Melting point (°C)	134-135	O'Neil (2001)
Vapour pressure (mm Hg)	6.29×10^{-7} at 25°C	Wiedemann (1972)
Henry's law constant ($atm \cdot m^3/mol$)	2.13×10^{-10}	EPISuite exp database
Water solubility	766 mg/L at 25°C	Seidell (1941)
Other solubilities (mg/L)	1 g dissolves in 1310 mL cold water, 82 mL boiling water, 15 mL cold alcohol, 2.8 mL boiling alcohol, 14 mL chloroform, 90 mL ether; soluble in glycerol.	O'Neil (2001)
Log K_{ow} (dimensionless)	1.58	Nakagawa et al. (1992)
Log K_{oc} (dimensionless)	1.699	Estimated PCKOCWIN v1.66
pK_a (dimensionless)	26.5	Estimated - Multicase

Abbreviations: K_{ow}, octanol–water partition coefficient; K_{oc}, organic carbon–water partition coefficient; pK_a, acid dissociation constant

4. Sources and uses

Phenacetin was included in a survey issued pursuant to section 71 of CEPA. For the 2008 calendar year, there were no reports of manufacture or import into Canada above the reporting threshold of 100 kg[4]. It was however reported as being imported into Canada, in quantities below or equal to the reporting threshold. Based on survey results for the 2008 calendar year, phenacetin is used in Canada as a laboratory substance (Environment Canada 2009), although this use is not expected to result in general population exposure.

Phenacetin is listed in the Natural Health Products Ingredients Database (NHPID) with a non-NHP role as it is present on the Prescription Drug List, as well as a homeopathic substance (e.g., as Phenacetinum). It is listed in the Licensed Natural Health Products Database (LNHPD) as being present in a limited number of homeopathic medicines licensed as natural health products (NHPID [modified 2018]; LNHPD [modified 2018]).

[4] Values reflect quantities reported in response to a survey conducted under section 71 of CEPA (Environment Canada 2009). See survey for specific inclusions and exclusions (schedule 2 and 3).

Based on notifications submitted under the Cosmetic Regulations to Health Canada, phenacetin was notified as being present in cosmetic products. Phenacetin is listed in the Personal Care Products Council's "International Nomenclature of Cosmetic Ingredients (INCI) Dictionary" with no stated function, although it is reported elsewhere to be used as a stabilizer for hydrogen peroxide (IARC, 2012). Product categories notified include: hair bleaches, hair colouring preparations, hair shampoos (colouring), and permanent waves (PCPC, 2016).

Phenacetin had a long history of use as an analgesic and antipyretic before being withdrawn from the market due to indications of nephropathy and increased risk of certain cancers in chronic, heavy users. In Canada, phenacetin was withdrawn from the market in June 1973 (Lexchin, 2005) although it remains on the Prescription Drug List for human and veterinary use (effective date 2013-12-19) (Health Canada, 2015). There are currently no marketed prescription drug products in Canada that contain phenacetin.

The U.S. Code of Federal Regulations Title 21 (21 CFR) indicates that drug products containing phenacetin were withdrawn from the U.S. market effective November 4, 1983 for reasons of safety and effectiveness (21 CFR 216.24) (US FDA, 1983). The basis of the withdrawal is "phenacetin's high potential for misuse and its unfavourable benefit-to-risk ratio when incorporated in analgesic combinations which are then subject to excessive chronic use."

5. Potential to cause ecological harm

5.1 Characterization of ecological risk

The ecological risk of phenacetin was characterized using the Ecological Risk Classification of organic substances (ERC) (ECCC 2016a). The ERC is a risk-based approach that employs multiple metrics for both hazard potency and exposure based on weighted consideration of multiple lines of evidence for determining risk classification. The various lines of evidence are combined to discriminate between substances of lower or higher potency and lower or higher potential for exposure in various media. This approach reduces the overall uncertainty with risk characterization compared to an approach that relies on a single metric in a single medium (e.g., LC_{50}) for characterization. Section 5 summarizes the approach, which is described in detail in ECCC (2016a).

Data on physical-chemical properties, fate (chemical half-lives in various media and biota, partition coefficients, fish bioconcentration), acute fish ecotoxicity, and chemical import or manufacture volume in Canada were collected from scientific literature, from available empirical databases (e.g., OECD QSAR Toolbox), and in response to surveys under section 71 of CEPA, or they were generated using selected Quantitative Structure-Activity Relationship (QSAR) or mass-balance fate and bioaccumulation models. These data were used as inputs to other mass-balance models or to complete the substance hazard and exposure profiles.

Hazard profiles were established based principally on metrics regarding mode of toxic action, chemical reactivity, food web-derived internal toxicity thresholds, bioavailability, and chemical and biological activity. Exposure profiles were also composed of multiple metrics including potential emission rate, overall persistence, and long-range transport potential. Hazard and exposure profiles were compared to decision criteria in order to classify the hazard and exposure potential for each organic substance as low, moderate, or high. Additional rules were applied (e.g., classification consistency, margin of exposure) to refine the preliminary classifications of hazard or exposure.

A risk matrix was used to assign a low, moderate or high classification of potential risk for each substance based on its hazard and exposure classifications. ERC classifications of potential risk were verified using a two-step approach. The first step adjusted the risk classification outcomes from moderate or high to low for substances which had a low estimated rate of emission to water after wastewater treatment, representing a low potential for exposure. The second step reviewed low risk potential classification outcomes using relatively conservative, local-scale (i.e., in the area immediately surrounding a point-source of discharge) risk scenarios, designed to be protective of the environment, to determine whether the classification of potential risk should be increased.

ERC uses a weighted approach to minimize the potential for both over and under classification of hazard and exposure and subsequent risk. The balanced approaches for dealing with uncertainties are described in greater detail in ECCC 2016a. The following describes two of the more substantial areas of uncertainty. Error with empirical or modeled acute toxicity values could result in changes in classification of hazard, particularly metrics relying on tissue residue values (i.e., mode of toxic action), many of which are predicted values from QSAR models. However, the impact of this error is mitigated by the fact that overestimation of median lethality will result in a conservative (protective) tissue residue used for critical body residue (CBR) analysis. Error with underestimation of acute toxicity will be mitigated through the use of other hazard metrics such as structural profiling of mode of action, reactivity and/or estrogen binding affinity. Changes or errors in chemical quantity could result in differences in classification of exposure as the exposure and risk classifications are highly sensitive to emission rate and use quantity. The ERC classifications thus reflect exposure and risk in Canada based on what is believed to be the current use quantity, and may not reflect future trends

Critical data and considerations used to develop the substance-specific profiles for phenacetin, and the hazard, exposure and risk classification results, are presented in ECCC (2016b).

Based on low hazard and low exposure classifications according to ERC for phenacetin, this substance was classified as having a low potential for ecological risk. It is therefore unlikely that these substances result in concerns for organisms or the broader integrity of the environment in Canada.

6. Potential to cause harm to human health

6.1 Exposure assessment

Between January 2013 and January 2016, phenacetin was notified in 11 cosmetics in Canada. All are hair colour products (1 temporary, 10 permanent) in which the ingredient is present at a concentration of 0.3% or less. Assuming a maximum phenacetin concentration of 0.3%, an area of skin exposed corresponding to the surface area of the adult scalp, and a retention coefficient of 10% to account for the fact that most of the product will not come in contact with skin when used as intended[5], a per event upper bounding surface load of 30 µg/cm^2 phenacetin was estimated (Table 6-1).

Table 6-1. Estimated upper bounding dermal surface load from the use of phenacetin-containing hair dye products.[6]

Product Scenario	Max. Conc. (%)	Frequency (x/year)	Exposed Area (cm^2)	Product Amount Applied (g)	Retention Coefficient (%)	Surface Load (external dose)
Hair dye application	0.3	12	565	50	10	30 µg/cm^2 per event

As no dermal penetration studies for phenacetin were identified, systemic exposure via the dermal route was estimated based on an established predictive algorithm to derive the maximum skin flux, or J_{max} (Williams et al., 2016). J_{max} is considered a conservative approach to estimating internal dose as the maximum flux at which a chemical can cross a unit area of skin (theoretically achieved as a saturated solution or in neat chemical form) defines the highest potential exposure risk for a chemical (IPCS, 2006). In order to estimate J_{max}, the model of Potts and Guy (1992) was used to calculate the skin permeability coefficient, kp (in cm h^{-1}) followed by the Cleek and Bunge (1993) modification (see Appendix A for details). J_{max} was determined to be 2.01 µg/cm²/h, yielding a per event systemic exposure of 0.011 mg/kg bw (Table 6-2). As phenacetin has a very low vapour pressure (6.29 x 10^{-7} mm Hg at 25°C), inhalation exposure during hair dye application is considered negligible relative to dermal exposure.

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[5] A retention factor of 10% for hair dyes was recommended by the SCCNFP (2000) to take into account rinsing off and dilution of finished products.

[6] The maximum concentration was determined based on notifications to Health Canada under the Cosmetic Regulations. Frequency, exposed area and product amount applied are from the RIVM Cosmetics Fact Sheet (RIVM, 2006). The retention coefficient is as recommended by the SCCNFP (2000).

Table 6-2. Estimated systemic exposure via the dermal route from the use of phenacetin-containing hair dye products.

Product Scenario	Assumptions[7]	Estimated Systemic Exposure
Hair dye application	Use of hair dye is considered episodic (approx. 12 times per year). **Dermal exposure:** Area of skin exposed = 565 cm^2, Duration of exposure = 40 min, J_{max} = 2.01 $\mu g/cm^2/h$, adult bw assumed to be 70.9 kg. It is assumed that gloves are used during application and skin contact only involves the scalp.	**Dermal per event:** 10.7 µg/kg bw **Dermal chronic:** 0.35 µg/kg bw/d

Empirical data on concentrations of phenacetin in environmental media in Canada were not identified, but are expected to be negligible. Phenacetin is not expected to be found in food or beverages.

Due to their nature as homeopathic medicines, exposure to the general population of Canada from the use of these products is expected to be minimal. The minimum homeopathic potency currently allowed in homeopathic medicines licensed as natural health products, based on the Homeopathic Pharmacopoeia of United States as outlined in the NHPID, is 6X, which is equivalent to a maximum concentration of approximately 10^{-6} g/mL (NHPID [modified 2018]).

6.2 Health effects assessment

6.2.1 Toxicokinetics

The metabolism of phenacetin has been well characterized in both humans and laboratory animals (see for example Brodie and Axelrod, 1949; Smith and Timbrell, 1974; Nelson et al., 1981; Hinson, 1983; Veronese et al., 1985; Fukami and Yokoi, 2012). First-pass metabolism is extensive, such that bioavailability of the parent compound is trivial via the oral route (Krieger Research Center, 2012). Metabolic pathways for phenacetin involve de-ethylation, N-deacetylation and ring hydroxylation. Although phenacetin is biotransformed to at least a dozen different metabolites, the main metabolic route is oxidative de-ethylation primarily by CYP1A2, giving rise to the pharmacologically-active metabolite n-acetyl-para-aminophenol (acetaminophen). In rats, rabbits, guinea pigs and ferrets administered 125 mg/kg bw phenacetin orally, 63, 57, 81 and 47% of the dose, respectively, was excreted as acetaminophen (free or

[7] Based on the RIVM Cosmetics Fact Sheet (RIVM, 2006) and Health Canada (1998).

conjugated as the sulfate or glucuronide) (IARC, 1980). In humans, it is estimated that 75 to 80% of orally administered phenacetin is rapidly metabolized to acetaminophen in normal individuals, with less than 1% of the parent compound excreted unchanged in the urine (Insel, 1993).

A secondary metabolic pathway for phenacetin involves hydrolysis to p-phenetidine by arylacetamide deacetylase (AADAC), a microsomal serine esterase expressed in liver and gastrointestinal tissues (Fukami and Yokoi, 2012). p-Phenetidine in turn may be further metabolized to the arylhydroxylamine metabolite N-hydroxyphenetidine, which is believed to be the proximate mutagenic metabolite that also mediates the nephro- and hematotoxicity of the parent compound. CYP1A2 has a much greater affinity for phenacetin than does AADAC (K_m = 31 µM for CYP1A2 versus 1.82 mM for AADAC) (Venkatakrishnan et al., 1998; Watanabe et al., 2010), although RNA expression in liver is similar for the two enzymes. Therefore, lower peak phenacetin levels would generally favour the high affinity metabolic pathway (CYP1A2), while high peak blood levels could favour a greater contribution by the low affinity pathway (AADAC). CYP1A2 follows Michaelis-Menten kinetics, which assumes a single binding site for the substrate at the active site of the enzyme, and metabolite formation following a hyperbolically saturating empirical model. The K_m is the concentration of substrate at which half the maximal reaction velocity (V_{max}, or the point at which CYP1A2 becomes saturated with phenacetin), is achieved. Canney and colleagues (1976) have shown that in normal volunteers, a 900-mg oral dose of phenacetin results in average peak plasma concentrations of phenacetin of 1,628 ng/ml (equivalent to 9.1 µM), which is well below the K_m of 31 µM, indicating this dose is insufficient to saturate the enzyme. It has been estimated that even at a substrate concentration of 100 µM, CYP1A2 would account for 86% of net reaction velocity (Venkatakrishnan et al., 1998). Other cytochrome P450 isoforms, such as CYP2E1, are also capable of oxidizing phenacetin, albeit with a lower affinity than CYP1A2.

Following oral administration of phenacetin in humans, peak plasma concentrations of acetaminophen derived from phenacetin de-ethylation occur in 1-2 hours (Insel, 1993). No data concerning the systemic availability of phenacetin via the dermal route were identified. Based on its physicochemical properties, including molecular weight, log $K_{o/w}$, and aqueous solubility, phenacetin is expected to be a "Medium High" penetrant according to the criteria of Kroes et al. (2007). Human skin, however, lacks significant expression of CYP1A2, the primary cytochrome P450 responsible for the metabolism of phenacetin via the oral route (Yengi et al., 2003), as well as AADAC, the enzyme that generates the toxic metabolite N-hydroxy p-phenetidine (Kobayashi et al., 2012). Therefore, because percutaneous exposure bypasses first pass metabolism, route-specific differences in toxicokinetics are anticipated.

Some individuals are recognized as poor metabolizers of phenacetin via CYP1A2. The incidence of this phenotype is expected to be less than 1% of the general population (Parkinson, 2001). There is also enormous inter-individual variability in CYP1A2 levels, and males tend to have higher levels than females. While genetic defects are extremely rare (Parkinson et al., 2013), individuals with limitations in the ability to metabolize

phenacetin to acetaminophen via CYP1A2 convert a greater fraction to the toxic arylhydroxylamine metabolite (Insel, 1993). Another potentially sensitive subpopulation is individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency. The red blood cells of patients with this enzyme defect are more susceptible to oxidative stress, and oxidant drugs such as phenacetin may therefore lead to acute or chronic hemolysis (WHO, 1989).

6.2.2 Acute toxicity

Phenacetin is of low to moderate acute oral toxicity in rats, with estimated LD_{50} values varying between 1650 and 4000 mg/kg bw (Boyd, 1959; Hart, 1947; Boyd and Hottenroth, 1968). Large but sublethal acute doses may cause methemoglobinemia and hemolytic anemia in humans and rats, although these endpoints are more generally associated with chronic overdosage (Jensen and Jollow, 1991; Gilman et al., 1990). The acute hemolytic anemia may be severe and accompanied by intravascular hemolysis, hemoglobinuria, and acute anuria, particularly in individuals with G6PD deficiency (de Leeuw et al., 1963). Phenacetin may also cause changes in energy and mentation, and is known to have mood-altering properties similar to caffeine which may contribute to its abuse liability (Margetts, 1976; Kincaid Smith, 1988).

No studies of acute phenacetin toxicity via the dermal route of exposure were identified.

6.2.3 Repeat-dose toxicity

Phenacetin was introduced into clinical medicine in 1887 and chronic overdose has long been associated with toxic effects, particularly of the hematopoietic and renal systems. An etiologic link between chronic phenacetin consumption and kidney disease began to emerge in Europe when, following the influenza pandemic of 1918, daily ingestion of phenacetin became routine for many individuals (Rennke and Denker, 2007). Erythrocyte damage, including methaemoglobin and Heinz body formation, as well as haemolytic anemia, were also recognized as common sequelae of prolonged phenacetin use or abuse (Brodie and Axelrod, 1949; Davidson, 1971). The first description of the nephropathy caused by chronic phenacetin intake was made by Spühler and Zollinger in 1953, who coined the term "primary chronic interstitial nephritis" to describe the characteristic renal lesions (as reported in Sanerkin and Weaver, 1964). The classic picture of phenacetin analgesic nephropathy includes medullary interstitial nephritis and fibrosis, papillary and proximal tubule damage, and chronic renal failure with loss of concentrating ability (HSDB, 2016).

Accurate estimates of the dosages of phenacetin that lead to analgesic nephropathy are difficult to establish, as they are largely based on patient recall over a period of years or decades, many of whom are not forthcoming. In the 1960s, Gault and co-workers (1968), while noting that only a small segment of the population grossly abuses analgesics, estimated the per capita annual consumption of phenacetin to be as high as 40 g in Australia, 25 g in Denmark, 23 g in Switzerland and 6-7 g in Canada. It has been estimated that decreased concentrating ability or a mild reduction in glomerular

filtration rate may be observed following cumulative intake of as little as 1 kg of phenacetin, whereas frank kidney disease requires a minimum intake of 2 to 3 kg, generally over a period of 6 to 8 years (Rennke and Denker, 2007). Therefore, a worst case estimate of 10 mg/kg bw/d phenacetin can be derived for the development of analgesic nephropathy (assuming an adult bw of 70.9 kg and ingestion of 2 kg over 8 years), although the uncertainty associated with this value is high.

Phenacetin toxicity has also been studied extensively in laboratory animals and the following description is not intended to be exhaustive, but rather focusses on the lowest published doses associated with toxicity. The lowest observed-effect level (LOEL) for phenacetin in repeat dose animal studies appears to be 350 mg/kg bw/d, based on reversible formation of methemoglobin and Heinz bodies and an increase in peripheral reticulocytes in rats following administration of 500 mg phenacetin per kg bw by oral gavage 5 times weekly for 4 weeks (Boelsterli et al., 1983). Animal models also show striking similarity to the renal functional changes associated with chronic analgesic use in humans (Bach and Hardy, 1985). Angervall and Bengtsson (1968) administered 450 mg/kg bw/d phenacetin to female SD rats in diet for 40 weeks, which they estimated to be "the highest possible dose which would not produce general toxic effects" (now referred to as the MTD or maximum tolerated dose). The dose used appeared to have a stimulatory effect similar to that observed in humans that take large doses, although the authors note that in animal studies using higher doses a depressive effect is observed. After 34 weeks, a decrease in urine concentrating capacity consistent with renal tubular impairment was observed, which was rapidly reversible upon discontinuation of the drug (Angervall and Bengtsson, 1968). More recently, in a study of toxicogenomic biomarkers for renal papillary injury in rats, Uehara and colleagues (2013) used phenacetin as a positive control. Male SD rats were administered phenacetin by oral gavage at 2000 mg/kg bw (single dose) or 1000 mg/kg bw/d (daily for 3, 7, 14, or 28 days), and kidney tissue was harvested and used for gene expression analysis 24-h after administration in the single-dose protocol and on days 4, 8, 15 and 29 of the repeat-dose study. Phenacetin-induced changes in genomic biomarkers associated with renal papillary injury occurred after a single dose and were observed one day following exposure, with histopathological changes apparent at four days post-dose.

No repeat-dose studies via the dermal route of exposure were identified.

6.2.4 Developmental and reproductive toxicity

There is limited evidence from animal studies to suggest that continuous exposure to phenacetin is associated with reproductive toxicity in rodents. Oral administration of phenacetin at doses of 600-1200 mg/kg bw/d from gestational days 0 to 20 is reportedly associated with reduced fetal weight, although the magnitude of the effect is not stated (Baethke and Muller, 1965 as cited in IARC, 1980). While there was no evidence of

teratogenicity, delayed skeletal growth and an increase in supernumerary ribs[8] was observed at doses of 150 mg/kg bw and above in the same study (no further details reported).

In humans, the U.S.-based Collaborative Perinatal Project monitored 5546 mother-child pairs with first trimester exposure to phenacetin (Briggs et al., 2011). No evidence suggested an association between phenacetin exposure in utero and large categories of major or minor malformations, although possible associations with some specific defects were noted: craniosynostosis (six cases); adrenal syndromes (five cases); anal atresia (seven cases); and accessory spleen (five cases). However, whether these associations are statistically significant is unknown and further independent confirmation is lacking (Briggs et al., 2011). Moreover, the fact that phenacetin was rarely used alone but rather in combination with other agents (usually acetylsalicylic acid and caffeine) further confounds interpretation of these results.

In a survey of 229,101 completed pregnancies in Michigan Medicaid recipients between 1985 and 1992, phenacetin exposure during the first trimester was reported in 368 cases. A total of 24 major birth defects were recorded versus 16 expected in this cohort and it was concluded that these data do not support an association between phenacetin exposure and congenital defects (Briggs et al., 2011). The U.S. FDA placed phenacetin in Pregnancy Risk Category B, which indicates animal reproduction studies have failed to demonstrate a risk to the fetus but there are no adequate and well-controlled studies in pregnant women.

6.2.5 Genotoxicity and carcinogenicity

Phenacetin is mutagenic in Salmonella typhimurium TA100 in the presence of liver 9000 g supernatant fractions (S9) from polychlorinated biphenyl (PCB)-treated hamsters but not rats (Nohmi et al., 1983). This is believed to be a result of species differences in deacetylation activity between rat and hamster liver microsomes, such that phenacetin is deacetylated to form direct-acting mutagens at rates 9 to 150 times greater in hamsters than in rats (Nohmi et al., 1983)[9]. Similarly, Camus and colleagues (1982) demonstrated that urine from phenacetin-treated hamsters but not rats is mutagenic in S. typhimurium TA100, and that N-hydroxyphenacetin is a proximate mutagenic metabolite of phenacetin following N-deacetylation.

There is also evidence that phenacetin causes chromosomal alterations or DNA damage in in vivo tests. A mouse micronucleus test indicated that relatively high doses

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[8] An increased incidence of supernumerary ribs is generally regarded as a nonspecific response to maternal factors (maternal toxicity and/or nonchemical stressors) and not sufficient evidence of a teratogenic effect in the absence of other indications.

[9] Human liver microsomes appear to be intermediate between rat and hamster, showing approximately 4- to 6.5-fold higher deacetylation activity than rat liver microsomes (Kobayashi et al., 2012).

of phenacetin (600 mg/kg bw and above) produced increases in micronuclei in bone-marrow erythrocytes, whether administered orally or intraperitoneally (Hayashi et al., 1989). Similar results were observed in rats administered phenacetin by oral gavage for 14 days at doses of 500 mg/kg bw and above (Asanami et al., 1995). In the gpt delta rat, a transgenic strain that possesses reporter genes for in vivo point mutations, 52-weeks of phenacetin treatment (0.5% in diet, estimated to be 202 and 246 mg/kg bw/d in males and females, respectively) induced an increase in gpt mutant frequency in the kidney of male, but not female rats, while no significant change in gpt mutant frequency was observed in either sex after 26-weeks (Kawamura et al., 2014). Although not direct evidence of genotoxicity, a dose-related increase in cellular proliferation in the urothelium of the bladder and kidney was observed in male SD rats exposed to phenacetin for 6 weeks in the diet at 1.0% and higher (Johansson et al., 1989). The induction of regenerative hyperplasia consequent to cytotoxicity is associated with an increase in the rate of mutation accumulation in the target organ and may influence tumour development.

In long-term carcinogenicity studies, phenacetin is a multi-sex, multi-site, multi-species carcinogen. In rats, it induces tumours in the kidney, nasal cavity, stomach and urinary bladder of males, and the ear/Zymbal's gland, mammary gland, nasal cavity and urinary bladder of females. In mice, target sites include the kidney in males and the urinary bladder in females (CPDB, 2007). The TD_{50}, or the daily dose to induce tumours in half of test animals that would have remained tumour-free at zero dose, is 1250 mg/kg bw/d in rats and 2140 mg/kg bw/d in mice (CPDB, 2007). In carcinogen risk assessment, a benchmark dose approach based on the lower 95% confidence limit on the dose that induces tumours in 10% of animals (the LTD_{10}) is generally used. A reliable estimate of the LTD_{10} can be derived using the TD_{50} and its lower 99% confidence limit according to the method of Gold and colleagues (2003). For phenacetin, the harmonic mean of LTD_{10} values from the most potent target site in each positive experiment in the Cancer Potency Database (CPDB) is 115 mg/kg bw/d in rats and 248 mg/kg bw/d in mice (CPDB, 2007).

Extrapolation from the LTD_{10} or other estimated dose near the lower limit of the observable range can also be used to derive a slope factor or unit risk factor, in order to estimate lifetime cancer risk. Using the BMDS 2.6 software (U.S. EPA, 2015) and the results of all tumour-bearing males, a multistage cancer model was fitted to the data of Isaka et al. (1979). This chronic dietary rat study was selected on account of its sensitivity in terms of adequate number of animals per group, adequate number of groups to model dose response, and the multiplicity of tissues examined for evidence of neoplastic transformation. The data from males only was modelled as they were more sensitive to the carcinogenic effects of phenacetin than females. The resulting $BMDL_{10}$ of 13.75 mg/kg bw/d (see Appendix B for details) is an order of magnitude lower than the LTD_{10} estimate based on the harmonic means of extrapolated TD_{50} values from all studies (both sexes) in the CPDB as described above. The cancer slope factor based on this model was determined to be 7.27 $[ug/kg/d]^{-1}$, which following allometric scaling to the ⅔ power of body weight corresponds to a human-equivalent value of 1.13 $[ug/kg/d]^{-1}$.

In the clinical and epidemiological literature, cases of renal pelvic and other urothelial tumours in patients who were heavy users of phenacetin-containing analgesics are well-documented, although phenacetin was generally used in combination with other analgesics, which makes it difficult to parse the contribution of phenacetin alone. Despite this limitation, a vast number of studies have been published which consistently suggest strong-to-moderate associations between regular use of phenacetin-containing analgesics and cancers of the renal pelvis and ureter (for review see IARC, 2012; Health Council of the Netherlands, 2012). In their evaluation of phenacetin, the IARC Working Group (2012) concluded as follows:

> "There is sufficient evidence in humans for the carcinogenicity of phenacetin. Phenacetin causes cancer of the renal pelvis, and of the ureter.
>
> There is sufficient evidence in experimental animals for the carcinogenicity of phenacetin.
>
> Phenacetin is carcinogenic to humans (Group 1).
>
> For the overall evaluation of phenacetin, the Working Group took into consideration that tumours of the renal pelvis and ureter are not known to result from the other components of the analgesic mixtures used in most countries; namely, aspirin, codeine phosphate, and caffeine."

It has been estimated that the total quantity of phenacetin taken by chronic heavy users ranged from 1.1 – 10.0 kg, with a latency period from beginning of the use to the diagnosis of the tumour averaging 24 years (Schmähl and Bunk, 1991). Thus, the chronic daily dose leading to tumour formation in humans can be roughly estimated as 1.8 – 16.1 mg/kg bw/d, although confidence in these estimates is low. Note that the usual dose of phenacetin as an over-the-counter remedy for pain and fever was 300 mg four to six times per day (IARC, 1977), which is equivalent to 16.9 – 25.4 mg/kg bw/d based on an adult bw of 70.9 kg.

6.3 Characterization of risk to human health

Consumer exposure to phenacetin is expected to be limited to the use of a small number of hair dye preparations, where the principle route of exposure is through dermal contact. As no suitable data from dermal studies were identified, a conservative estimate of systemic dose via the dermal route was derived using the predicted maximum flux, which defines the theoretical highest exposure potential attainable for a given chemical. The per event systemic exposure was estimated to be 0.011 mg/kg bw. Hair dye products are estimated to be used up to 12 times per year, leading to a dose-averaged chronic exposure of 0.00035 mg/kg bw/d.

Based principally on the assessments of the International Agency for Research on Cancer (IARC, 2012), the U.S. EPA (2002) and the U.S. NTP (2014), the critical effect for characterization of risk to human health for phenacetin is carcinogenicity.

Phenacetin is carcinogenic to humans and animals and although the mechanism of induction for the tumours has not been fully elucidated, the available evidence indicates this substance or its metabolites may have genotoxic potential. The rat appears to be more sensitive than the mouse and males appear more sensitive than females. Therefore, the critical effect level was determined to be the lower 95% confidence limit on the benchmark dose ($BMDL_{10}$) equal to a 10% increase in the incidence of all tumour types in treated male rats relative to controls in the study of Isaka et al. (1979). Thus, the point of departure for risk characterization is the rat $BMDL_{10}$ of 13.75 mg/kg bw/d.

Comparison of the chronic systemic exposure level from use of hair dye with the rat oral $BMDL_{10}$ yields an MOE greater than 39,000 (Table 6-3), which indicates a low level of concern. While the MOE is a useful approach to characterize the magnitude of a risk, it cannot be used to directly quantify the increased probability of an adverse health effect. Therefore, a human cancer potency value for phenacetin was also derived using a multistage cancer model and allometric scaling. The oral slope factor of 1.13 $[ug/kg/d]^{-1}$ can be used to calculate the incremental lifetime cancer risk of exposure to phenacetin through the use of hair dye products (see Appendix B). The carcinogenic risk is estimated to be 4.6×10^{-7}, which is widely regarded as negligible.

With respect to non-cancer endpoints, nephropathy and hematotoxicity have also been associated with both prolonged exposure to phenacetin as well as large acute doses. The lowest LOEL for phenacetin from repeat dose animal studies appears to be 350 mg/kg bw/d, based on reversible formation of methemoglobin and Heinz bodies and an increase in peripheral reticulocytes in rats following administration by oral gavage for 4 weeks (Boelsterli et al., 1983). A comparison of this critical effect level with the estimated per event systemic dose of 0.0107 mg/kg bw/d from the use of phenacetin-containing hair preparations results in an MOE for short-term exposure of approximately 33,000 (Table 6-3).

Table 6-3. Upper-bounding estimates of exposure and resulting margins of exposure.

Product	Estimated Systemic Exposure	Critical Effect Level	Critical Hazard Endpoint	MOE
Hair dye preparation (chronic)	0.35 µg/kg bw/d	13.75 mg/kg bw/d (The $BMDL_{10}$ for all tumour-bearing males from Isaka et al., 1979)	Carcinogenicity	~39,000

Hair dye preparation (per event)	10.7 µg/kg bw	350 mg/kg bw/d (LOAEL based on 4-wk rat oral gavage study).	Reversible formation of methemoglobin and Heinz bodies and an increase in peripheral reticulocytes.	~33,000

The margins between upper-bounding estimates of exposure and critical effect levels observed in animal studies are considered adequate to account for both cancer and non-cancer effects and any uncertainties in the toxicological and exposure databases.

While exposure of the general population to phenacetin is not of concern at current levels, this substance is considered to have a health effect of concern based on its carcinogenic potential. Therefore, there may be a concern for human health if exposure were to increase.

6.4 Uncertainties in evaluation of risk to human health

Confidence in the exposure database is considered moderate, as although Canadian data were available on cosmetics to allow the derivation of upper-bounding exposure estimates, no experimental dermal absorption data were identified for phenacetin, and systemic exposure via the dermal route was estimated using a predictive algorithm for skin permeability. Confidence in the hazard database is high, as the adverse effects associated with exposure to this substance are extensively documented.

There is uncertainty concerning the scientific validity of the oral-to-dermal route extrapolation. On account of its short half-life and the rate and extent of pre-systemic metabolism following oral exposure, phenacetin is not an ideal candidate for extrapolating toxicity from the enteral to parenteral routes. However, the extrapolation is considered highly conservative, as the toxic metabolite(s) are a product of the first-pass effect; this source of uncertainty therefore does not detract from confidence in the conclusion. Even considering equivalent internal dosimetry in terms of area under the curve, the peak concentration (C_{max}) of the reactive metabolite(s) is expected to be lower via the dermal route and thus less likely to saturate detoxification or DNA repair mechanisms.

Lastly, there is uncertainty regarding the use of dose averaging to amortize doses received intermittently over a period of chronic exposure, particularly for a substance with a relatively short biological half-life. The principle of dose averaging is based on Haber's rule and the assumption that toxicity is related to the total combined exposure. While the basic concept is routinely applied in cancer risk assessment for genotoxic carcinogens, there is uncertainty as to the extent which average exposure calculated by dose averaging reflects the relevant measure of exposure in toxicological terms. However, the comparison of effect levels from laboratory studies involving chronic

exposure over the course of a lifetime with brief, intermittent exposure in humans is considered highly conservative.

7. Conclusion

Considering all available lines of evidence presented in this screening assessment, there is low risk of harm to organisms and the broader integrity of the environment from phenacetin. It is therefore concluded that phenacetin does not meet the criteria under paragraphs 64(a) or (b) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

Based on the information presented in this Screening Assessment, it is concluded that phenacetin does not meet the criteria under paragraph 64(c) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

Therefore, it is concluded that phenacetin does not meet any of the criteria set out in section 64 of CEPA.

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Appendix A – Upper-bounding estimated exposure from use of hair dye products

A-1 Calculation of systemic exposure based on maximum flux.

The model of Potts and Guy (1992) calculates the skin permeability coefficient (K_p) (in cm/h) based on permeant size (expressed as molecular weight) and lipophilicity (expressed as the logarithm of the octanol:water partition coefficient). Given that log $K_{o/w}$ = 1.58 and molecular mass (weight) of phenacetin is 179.2 g/mole, log K_p is calculated as:

$$\log K_p \text{ (cm/h)} = -2.72 + 0.71 \log K_{o/w} - 0.0061 * MW$$

$$\log K_p \text{ (cm/h)} = -2.691$$

$$K_p \text{ (cm/h)} = 2.03 \times 10^{-3}$$

The Cleek and Bunge (1993) correction is then applied to account for the relative permeabilities of the stratum corneum and the epidermis:

$$K_{p,}\text{mod (cm/h)} = K_p / \{1 + (K_p * \sqrt{MW}) / 2.6\}$$

$$K_{p,}\text{mod (cm/h)} = 2.01 \times 10^{-3}$$

The maximum flux (J_{max}) can then be calculated from the modified skin permeability coefficient and the aqueous solubility of the compound ($C_{sat} \approx 1$ mg/mL) as follows:

$$J_{max} \text{ (µg/cm}^2\text{/h)} = 1000 \text{ µg/mg} * K_{p,}\text{mod (cm/h)} * C_{sat} \text{ (mg/cm3)}$$

$$J_{max} \text{ (µg/cm}^2\text{/h)} = 2.01$$

Using the predicted maximum flux, surface area of exposure and duration of exposure, the maximum systemic dose may be estimated.

Table A-1: 9.1 Calculation of systemic exposure based on maximum flux

Scenario	Model Parameters[10]	Estimated Exposure
Hair Dye	- Exposure frequency: 12/year - Body weight: 70.9 kg - Surface area of exposure: 565 cm^2	**Dermal per event:** 0.013 mg/kg bw

[10] Assumptions are based on RIVM (2006) and Health Canada (1998).

	- Duration of exposure: 40 min	**Dermal chronic:** 0.00041 mg/kg bw/d

Systemic exposure dose per event = 2.01 µg/cm^2/h * 565 cm^{2*} 40/60 h / 70.9 kg

Chronic systemic dose = per event dose * 12/365

Appendix B - Calculation of carcinogenic risk

B-1 Derivation of the $BMDL_{10}$ for phenacetin

Benchmark dose calculations were performed using the BMDS 2.6 software (U.S. EPA). The multistage cancer model was fitted to the data of Isaka et al. (1979) using all instances of tumours in males that were determined to be "effective animals" (Table 9-1). The authors defined an effective animal as one that survived more than 24 months or died due to tumours that developed within 24 months. A bench mark response (BMR) equal to a 10% increase in tumour incidence relative to controls ($BMD_{0.1}$) was derived along with its 95% lower confidence limits ($BMDL_{10}$).

Table B-1. Model input data (from Isaka et al., 1979).

Dose (mg/kg bw/d)	Total number of animals	Number of tumour-bearing animals
0	19	1
365	22	20
750	27	26

The multistage cancer model is the default model used by the US EPA for cancer bioassay data. Although other models are available for fitting dichotomous data, none offered either greater conservatism or improved goodness of fit (Table 9-2). Therefore, the $BMDL_{10}$ of 13.75 mg/kg bw/d from the multistage cancer model was selected as the point of departure for risk assessment (Figure 9-1).

Table B-2. BMDs and goodness of fit for available dichotomous models.

Model	$BMD_{0.1}$	$BMDL_{10}$	chi-square	p-value	AIC[1]	Residuals[2]
Gamma	19.74	13.75	0.99	0.3207	34.67	-0.8 to 0.6
Logistic	72.05	44.03	8.75	0.0031	38.29	-2.7 to 1.0
Multistage-cancer	19.74	13.75	0.99	0.3207	34.67	-0.8 to 0.6
Probit	67.81	46.54	11.11	0.0009	40.96	-2.7 to 1.6
Weinbull	19.74	13.75	0.99	0.3207	34.67	-0.8 to 0.6
Quantal-Linear	19.74	13.75	0.99	0.3207	34.67	-0.8 to 0.6

[1] AIC is the Akaike information criterion, defined as AIC= -2 x (LL-p), where LL is the log-likelihood at the maximum likelihood estimates and p is the degrees of freedom. All else being equal, a lower AIC is preferred.

[2] [(Observed value – expected value)/standard error]

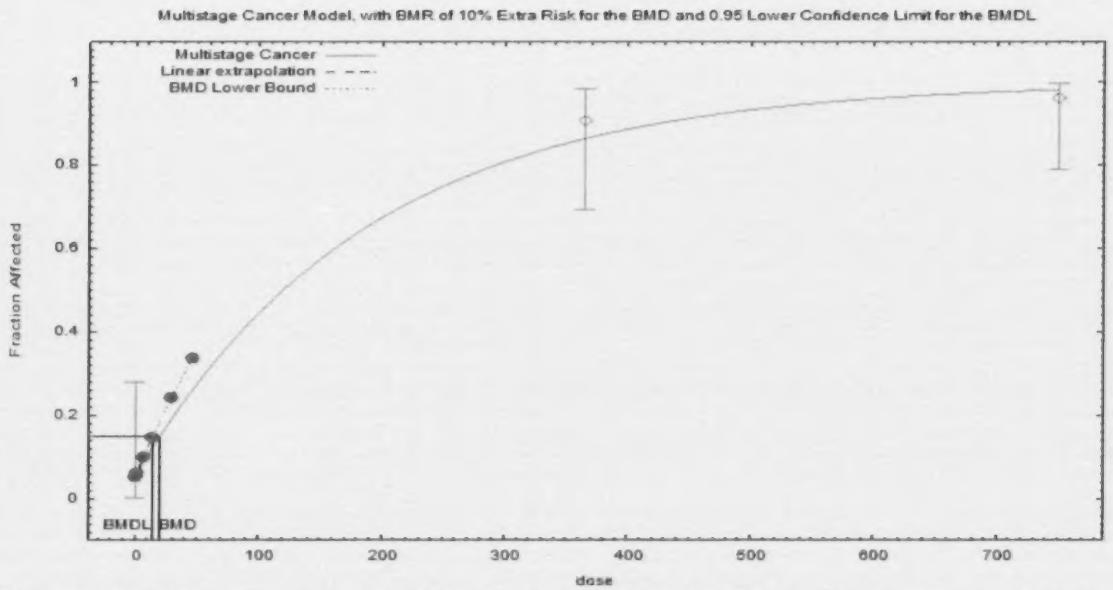

Figure B-1. Multistage cancer model fitted to the combined data from all tumour-bearing males in Isaka et al. (1979).

B-2 Estimated incremental lifetime cancer risk based on carcinogenic potency of phenacetin

Cancer potency is proportional to the slope of the dose response curve at low doses. A multistage cancer model was fitted to the animal bioassay data of Isaka et al. (1979). There is an inherent assumption in this approach that data collected at high doses are also relevant at very low doses, or that the model is capable of extrapolating potency outside the range of experimental observations to yield estimates of "low" dose potency (Cal/EPA, 1992).

To estimate cancer potency, the Benchmark response (BMR) of 0.1 is divided by the lower 95% confidence limit on the dose that induces tumors in 10% of animals ($BMDL_{10}$).

Cancer slope factor = BMR/ $BMDL_{10}$ = 0.1/13.75 mg/kg bw/d = 7.27 $[ug/kg/d]^{-1}$

This cancer slope factor derived from the bioassay data may be allometrically scaled by the 2/3 power of body weight to yield a human-equivalent slope factor:

Human equivalent slope factor = animal slope factor $[ug/kg/d]^{-1}$ × (bw animal / bw human) ^ (1- b)

, where b = 0.667 (⅔ power scaling)

∴ human equivalent slope factor = 7.27 $[ug/kg/d]^{-1}$ × (0.267 kg / 70.9 kg) ^ 0.333 = 1.13 $[ug/kg/d]^{-1}$

This value may be multiplied by the chronic exposure dose in order to derive an estimate of incremental lifetime cancer risk.

$$0.00113\ [mg/kg/d]^{-1} \times 0.00041\ mg/kg\ bw/d = 4.6 \times 10^{-7}$$